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Growth on Octane Alters the Membrane Lipid Fatty Acids of *Pseudomonas oleovorans* due to the Induction of *alkB* and Synthesis of Octanol

QI CHEN, DICK B. JANSSEN, AND BERNARD WITHOLT*

Department of Biochemistry, University of Groningen, 9747 AG Groningen, The Netherlands

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Growth of *Pseudomonas oleovorans* GPo1, which contains the OCT plasmid, on octane results in changes in the membrane phospholipid fatty acid composition. These changes were not found for GPo12, an OCT-plasmid-cured variant of GPo1, during growth in the presence or absence of octane, implying the involvement of OCT-plasmid-encoded functions. When recombinant strain GPo12(pGEc47) carrying the *alk* genes from the OCT plasmid was grown on octane, the cells showed the same changes in fatty acid composition as those found for GPo1, indicating that such changes result from induction and expression of the *alk* genes. This finding was corroborated by inducing GPo12(pGEc47) with dicyclopropylketone (DCPK), a gratuitous inducer of the *alk* genes. Further experiments showed that the increase of the mean acyl chain length of fatty acids is related to the expression of *alkB*, which encodes a major integral membrane protein, while the formation of *trans* unsaturated fatty acids mainly results from the effects of 1-octanol, an octane oxidation product.

Pseudomonas oleovorans is able to use *n*-alkanes with 6 to 12 carbon atoms as the sole carbon and energy sources (1). The cells can be grown in two-liquid-phase medium, composed of an aqueous phase and a bulk alkane phase at volume fractions from 5 to 95% (vol/vol) *n*-octane (28). The enzymes that convert *n*-alkanes to acyl coenzyme A are encoded by the *alk* genes, which are located on the OCT plasmid (Fig. 1) (10–13, 26, 37). The AlkB component of the alkane hydroxylase is a major membrane protein, which accounts for 25 to 30% of the total cytoplasmic membrane protein content of *P. oleovorans* (12, 28).

When grown in a two-liquid-phase medium, containing a bulk *n*-octane phase, the cellular membranes of *P. oleovorans* are altered, as seen by freeze-fracture electron microscopy (8, 34), by lipid fatty acid analysis, and by membrane fluidity measurements (4). In particular, the membrane fatty acid composition changes: more 18:1 fatty acids, more unsaturated fatty acids, and *trans* unsaturated fatty acids are formed. As a result of these changes, the transition temperature of the membrane lipid increases by about 20°C (4), which compensates for the potential bilayer fluidizing effects of *n*-octane (30).

When *P. oleovorans* is grown on *n*-octane in two-liquid-phase medium, the cells are exposed to a bulk octane phase. In addition, the *alk* genes are induced, and as a consequence, *n*-octane is oxidized. Since both organic solvents and membrane proteins can influence membrane lipid composition and properties (21, 30, 40, 41), two alternative hypotheses can explain the above effects. First, membrane lipid changes are only a general effect of exposure of *P. oleovorans* to *n*-octane. Second, membrane lipid changes are related specifically to the presence and activity of the *alk* genes in *P. oleovorans.*

To test these two hypotheses, we have compared the effects of growth in (or on) octane and the effects of inducing the *alk* genes on the fatty acid composition of three types of *P. oleovorans* strains: GPo1, the wild-type strain which harbors the natural catabolic OCT plasmid; GPo12, which is an OCT-plasmid-cured GPo1 variant; and recombinant strains which were constructed by introducing different *alk* genes derived from the OCT plasmid into GPo12.

In this paper, we describe experiments for which the results eliminate the first hypothesis. Our data clearly show that the changes in membrane lipid fatty acid composition required the induction of the *alk* genes. The expression of *alkB* and formation of 1-octanol from *n*-octane each contributed to specific changes in the fatty acid composition during the growth of *P. oleovorans* on octane.

MATERIALS AND METHODS

Strains and plasmids. *P. oleovorans* wild-type strain GPo1 and its OCT-plasmid-cured variant, GPo12, were used throughout the experiments. The OCT plasmid was cured from *P. oleovorans* by NTG (nitrosoguanidine) mutagenesis as described by Kok (26). Attempts to cure the plasmid under nonselective conditions were unsuccessful. Therefore, a series of alkane$^-$ and alkanol$^+$ mutants of *P. oleovorans* were isolated, and colony hybridization with an *alkBFGH* probe was used to select a dozen strains which lacked the *alkBFGH* genes. Each of these strains lacked the entire OCT plasmid, as shown by the fact that they could stably maintain the antibiotic resistance plasmid R3108 (15). Plasmid R3108 and the OCT plasmid do not coexist in one host under nonselective conditions because they belong to the same incompatibility IncP-2 group (3, 15). GPo12 is one such mutant. To confirm that the OCT plasmid was cured, GPo12 was tested for Hg resistance (an OCT phenotype) and found to be Hg^s (26). The plasmids listed in Table 1 were introduced into GPo12 according to the method of Ditta et al. (9) to generate different recombinants.

Medium, growth conditions, and induction of *alk* genes with DCPK (dicyclopropylketone). The cells were precultured at 30°C in 250-ml Erlenmeyer flasks containing 50 ml of medium. For octane cultivation, the preculture was grown in E medium (39) supplemented with 0.1% (vol/vol) MT microelements (29) and 2% (vol/vol) octane. For the citrate cultivation, the preculture was grown in E_2 medium (29) with 2.1% (wt/vol) trisodium citrate dihydrate.

DCPK (0.05% [vol/vol]) was used to induce the *alk* genes when *P. oleovorans* strains were grown on citrate. DCPK is a gratuitous *alk* inducer which cannot be used as a growth substrate or a respiration substrate by *P. oleovorans* (18). We found that DCPK had no effect on the growth of GPo12, which does not contain *alk* genes. When wild-type strain GPo1 containing the *alk* genes was grown on citrate, DCPK reduced the exponential growth rate (μ) to about 50% of that seen in the absence of DCPK, and the final cell density in the stationary phase was lowered to 77% of that found in the absence of DCPK. This was due to induction of the *alk* genes; DCPK has no effect on the growth of the wild-type strain GPo1 grown on octane, in which the *alk* genes were already switched on by octane, indicating that DCPK had no effects on the growth of *P. oleovorans*

* Corresponding author. Present address: Institut für Biotechnologie, ETH Hönggerberg, HPT CH 8093 Zürich, Switzerland. Phone: 41-1-6332088. Fax: 41-1-6331051.

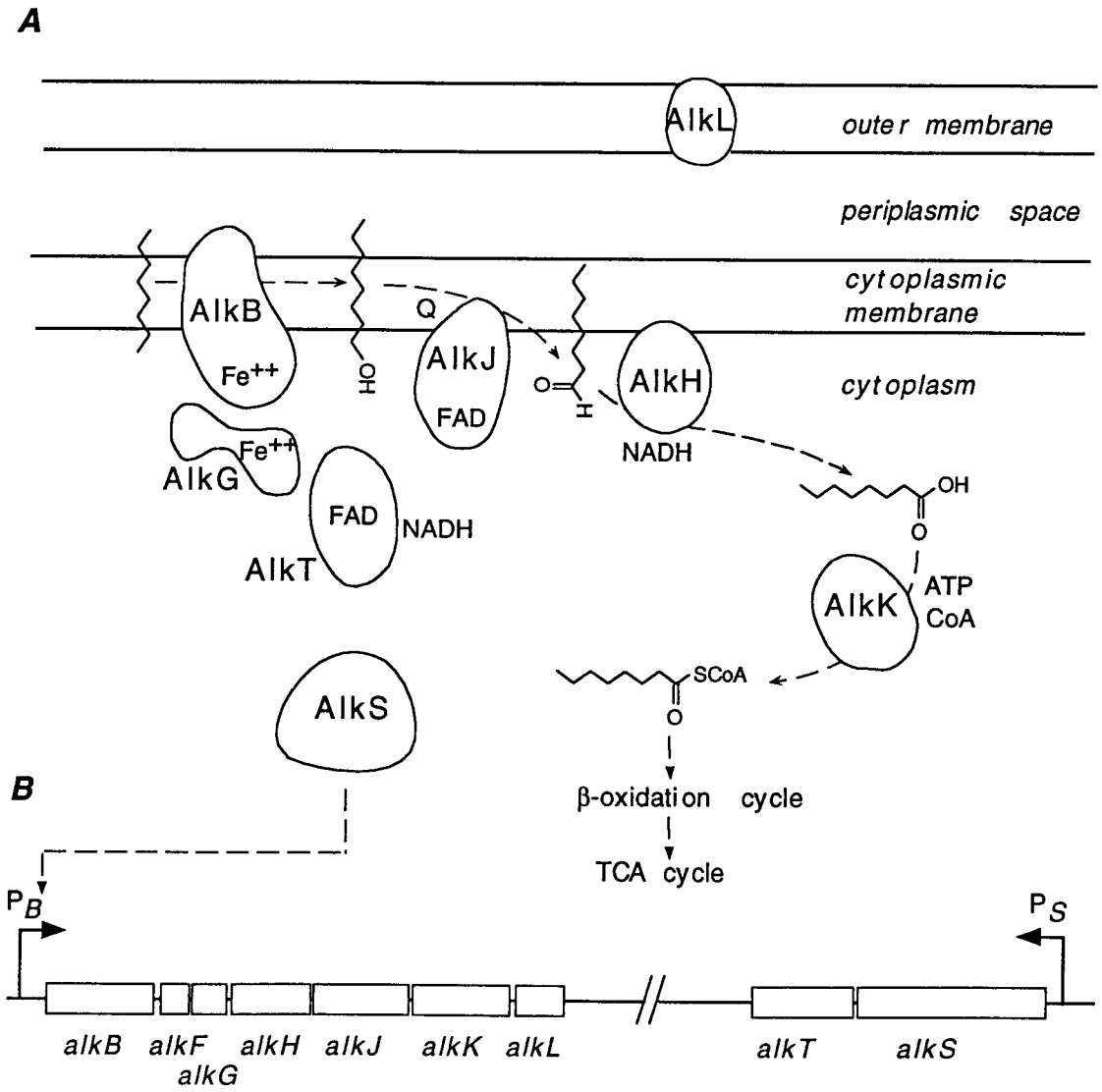

FIG. 1. Alkane oxidation system encoded by OCT plasmid of *P. oleovorans*. (A) Functions and cellular localization of Alk proteins; (B) organization of *alk* genes. The expression of *alkBFGHJKL* operon is positively regulated by AlkS. AlkB, alkane hydroxylase; AlkG, rubredoxin; AlkH, aldehyde dehydrogenase; AlkJ, alcohol dehydrogenase; Q, coenzyme Q; AlkK, acyl coenzyme A (CoA) synthetase; AlkS, regulatory protein; AlkT, rubredoxin reductase; TCA, trichloroacetic acid; FAD, flavin adenine dinucleotide.

other than those resulting from the induction of the *alk* genes. Similar effects have been observed in *Escherichia coli alk*$^+$ recombinants (14).

Continuous cultures were carried out as described previously (4) except for experiments in which the *alk* genes were induced with DCPK. Here, 1.1% (wt/vol) instead of 2.1% (wt/vol) trisodium citrate dihydrate was used since this concentration led to higher AlkB expression levels in batch cultures at 24 h after inoculation. Because nitrogen was added in limiting amounts in the continuous cultures (4), the medium was diluted 2.5-fold to achieve the desired (lower) nitrogen level, so that the salt components were present at the following concentrations (per liter): 0.4 g of K_2HPO_4, 0.44 g of $(NH_4)_2SO_4$, 0.246 g of $MgSO_4 \cdot 7H_2O$, 1 ml of modified MT microelements (34). Correspondingly, precultures were grown in the 2.5-fold diluted E_2 medium with 0.5% (wt/vol) trisodium citrate dihydrate. In the case of recombinant strains, 12.5 mg of tetracycline per liter was added to the medium for positive selection of the plasmids when cells were grown on citrate.

TABLE 1. List of plasmids used in this study

Plasmid	Relevant characteristic(s)	Reference
pLAFRI	Tc^r, Tra, Mob, RK2 replicon	16
pGEc47	pLAFRI, *alkST/alkBFGHJKL*	12
pGEc47Δ*B*	pGEc47, 528-bp *Bam*HI deletion in *alkB*	38
pGEc74	pLAFRI, *alkST*	10
pGEc29	pLAFRI, *alkBFGHJKL*	10

The cell density was determined at an optical density at 450 nm (42). Samples were collected from the continuous cultures between three to five residence times after a new dilution rate was set, during which time the culture generally reached a steady state.

Detection of *alk* expression. The expression of the *alk* genes was estimated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of total cellular proteins (27), in which AlkB was present as an obvious band in the gel after staining with Coomassie blue. Whether the *alk* genes were still active was determined by growing cells on minimal salt medium plates in a closed container saturated with gaseous *n*-octane.

Determination of phospolipid fatty acid. The fatty acids were methylated and analyzed by gas chromatography as described previously (4). In preliminary experiments, we cultivated wild-type strain GPo1 in continuous culture at several dilution rates, using either octane or citrate as the carbon source. The total membranes were isolated, and the membrane lipids were extracted for the analysis of fatty acid composition. We also analyzed fatty acids from the corresponding whole cells which contained additional fatty acids, such as lipopolysaccharide-associated fatty acids. However, there were no differences in the relative

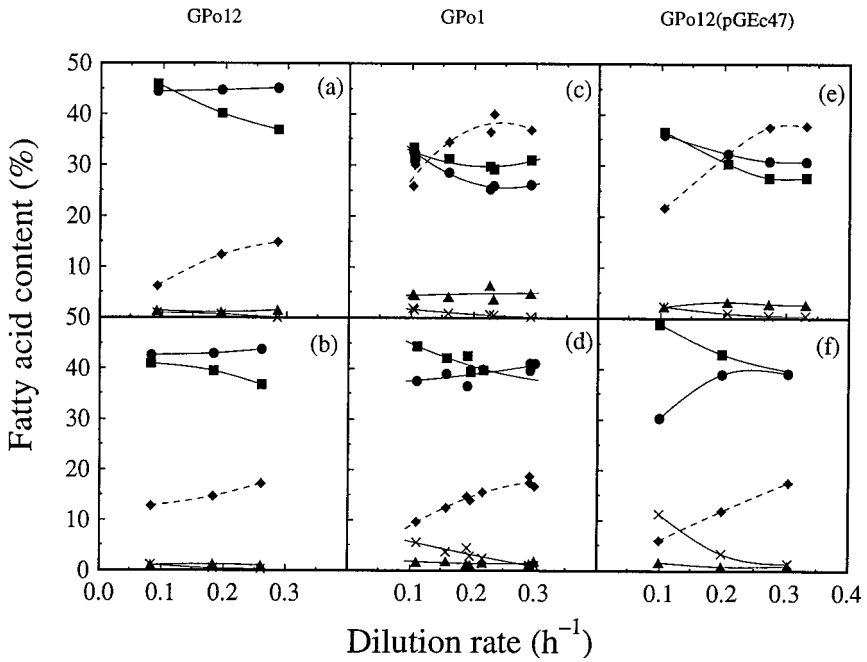

FIG. 2. Fatty acid composition of *P. oleovorans* GPo12 (OCT^-), GPo1 (OCT^+), and recombinant strain GPo12(pGEc47) (alk^+) at different growth rates in continuous cultures. The cells were grown in a 1-liter stirred tank reactor containing minimal salts medium supplemented with either 15% (vol/vol) octane or 2.1% trisodium citrate dihydrate as the carbon source. Fatty acid 14:0 accounted for less than 1% and is not shown in the figure. GPo12 was grown on citrate in the presence (a) or absence (b) of 15% (vol/vol) octane, GPo1 was grown on octane (c) or on citrate (d), and GPo12(pGEc47) was grown on octane (e) or on citrate (f). ■, 16:0; ●, 16:1; ×, 17:cy; ▲, 18:0; ◆, 18:1.

molar compositions of the set of fatty acids derived from extracted lipids and the corresponding fatty acids from whole cells. This was true for cells grown on citrate and on octane, in continuous cultures at three different dilution rates. Therefore, the membrane lipid fatty acid composition was routinely determined in whole cells. The fatty acid composition was expressed as a percentage (mole/mole) of the total set of membrane lipid fatty acids.

Exposure of GPo12 to 1-octanol. GPo12 was grown in 250-ml Erlenmeyer flasks containing 50 ml of E_2 medium, with 1% sodium citrate dihydrate as the carbon source. The cells were harvested at a cell density of about 0.5 mg/ml and suspended in the same medium to the same cell density. After incubation at 30°C for 30 min, the organic solvents were added to the culture, and the fatty acid composition was monitored at regular intervals.

RESULTS

Effects of octane on fatty acid composition of *P. oleovorans* GPo12 and GPo12(pGEc47) in continuous culture. In our preliminary experiments, we found that growth of wild-type strain

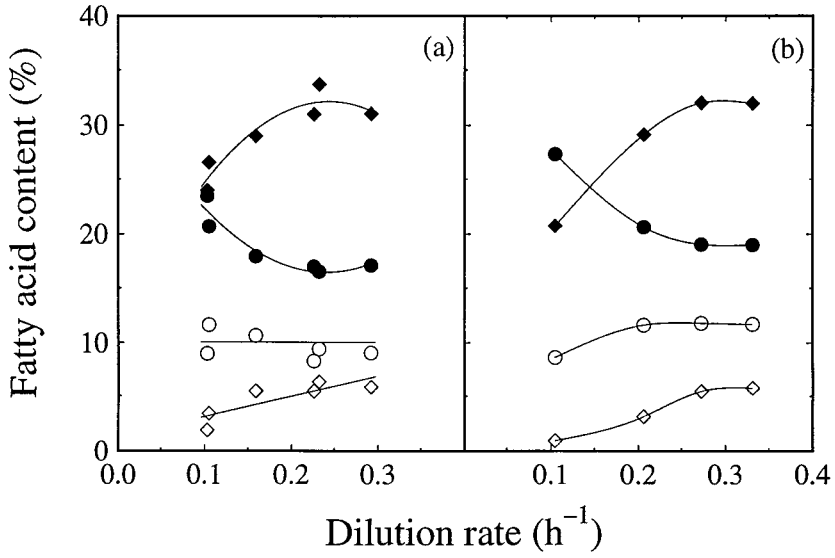

FIG. 3. Formation of *trans* and *cis* unsaturated fatty acids by *P. oleovorans* GPo1 (OCT^+) (wild-type strain) (a) and GPo12(pGEc47) (alk^+) (recombinant strain) (b) during growth on 15% (vol/vol) octane in continuous cultures described in the legend to Fig. 2c and e. ●, 16:1(9c); ○, 16:1(9t); ◆, 18:1(11c); ◇, 18:1(11t).

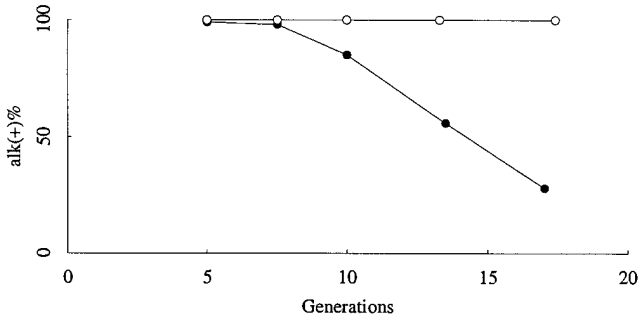

FIG. 4. Stability of *alk* genes of GPo12(pGEc47) during continuous growth in minimal medium containing 1.1% (wt/vol) trisodium citrate dihydrate at a dilution rate of about 0.1 h^{-1} in the presence (●) or absence (○) of DCPK.

GPo1 on octane in batch culture resulted in changes in both the phospholipid fatty acid composition and the phospolipid composition. The changes in the phospholipids were minor: phosphatidylethanolamine increased from 79 to 83% and phosphatidylglycerol decreased from 18 to 13% of the total phospholipids. The changes in the fatty acid composition were significant, and we monitored such changes in more detail in continuous cultures. The fatty acid compositions of *P. oleovorans* GPo12, in which the OCT plasmid is absent, and GPo12(pGEc47), an *alk*$^+$ recombinant, were compared with that of wild-type strain GPo1, during growth in the presence or absence of *n*-octane at different dilution rates.

The fatty acids found in the membrane lipids of *P. oleovorans* include tetradecanoic acid (14:0), hexadecanoic acid (16:0), 9-*cis*-hexadecenoic acid [16:1(9c)], 9-*trans*-hexadecenoic acid [16:1(9t)], *cis*-9,10-methylene-hexadecanoic acid (17:cy), octadecanoic acid (18:0), and 11-*cis*-octadecenoic acid [18:1(11c)]. Figure 2a and b show that the fatty acid profiles of GPo12 grown on citrate in the presence or absence of 15% (vol/vol) octane were similar, indicating that octane did not influence the fatty acid composition of the membrane

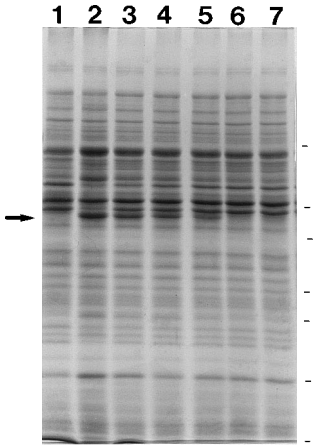

FIG. 5. Expression of *alkB* in GPo12(pGEc47) during continuous growth in aqueous medium with citrate as carbon source in the presence of DCPK. Samples were taken from the culture after the number of generations shown in Fig. 4, and proteins from total cell lysates were analyzed by SDS-PAGE (12.5% acrylamide). Lanes: 1, uninduced GPo12(pGEc47); 2 to 7, GPo12(pGEc47) after induction for 5, 7.5, 10, 13.5, 17, and 20 generations, respectively. The arrow indicates the AlkB band. The sizes of the molecular mass standards (indicated by horizontal lines on the right) are 66, 45, 36, 29, 24, 20, and 14 kDa, respectively (from top to bottom).

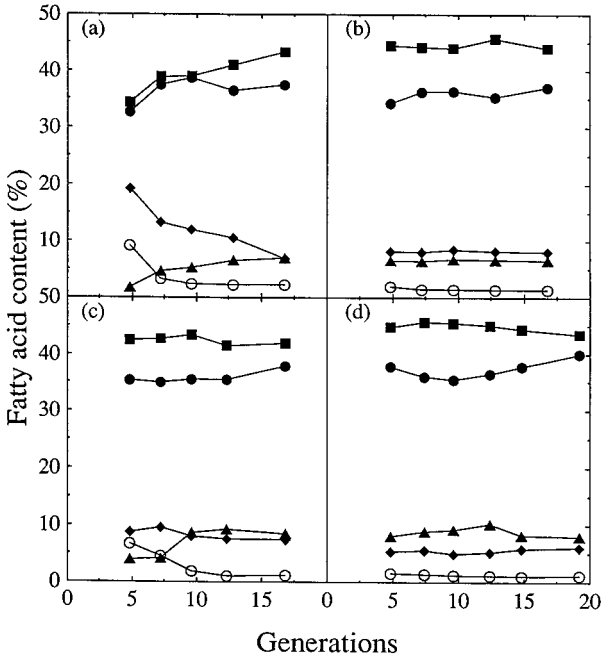

FIG. 6. Effects of induction of *alk* genes with DCPK on fatty acid composition. GPo12(pGEc47) (a and b) and GPo12(pGEc47Δ*B*) (c and d) were grown continuously in minimal medium containing 1.1% (wt/vol) trisodium citrate dihydrate at a dilution rate of about 0.1 h^{-1}, in the presence (a and c) or absence (b and d) of DCPK. ■, 16:0; ●, 16:1(9c); ○, 16:1(9t); ▲, 17:cy; ◆, 18:1(11c).

phospholipids of GPo12. The profile shown in Fig. 2a and b is referred to below as the standard fatty acid profile.

Similar experiments for the wild-type strain *P. oleovorans* GPo1 (4) resulted in the standard fatty acid profile for cells grown on citrate (Fig. 2d). However, during growth on octane, a different fatty acid profile at changing dilution rates was obtained (Fig. 2c) and is referred to hereafter as the octane-induced profile. The prominent fatty acid, 18:1, increased from 26% at low dilution rates to a maximum of 37%, while 16:0 and 16:1 fatty acids were present at only about 30% each.

Differences between the octane-induced and the standard profiles were also reflected in the *trans* versus *cis* unsaturated fatty acid compositions. Octane-grown GPo1 (Fig. 3a) contained about 10% (16:1(9t), while 11-*trans*-octadecenoic acid [18:1(11t)] increased from 2 to 8% as the dilution rate increased. In contrast, GPo1 contained less than 3% *trans* unsaturated fatty acids during growth on citrate. GPo12 produced equally small amounts of *trans* unsaturated fatty acids either in the absence or presence of octane. The level of 16:1(9t) never exceeded 4%, and 18:1(11t) was not detected (data not shown).

Since 15% (vol/vol) octane had little effect on *P. oleovorans* GPo12, the octane-induced profile found for octane-grown GPo1 must be related to the presence of the OCT plasmid and might well be associated with the *alk* genes. To test this possibility, plasmid pGEc47, which contains two OCT-plasmid-derived fragments that carry the *alk* genes cloned into the broad-host-range vector pLAFRI (10), was introduced into GPo12.

Continuous culturing of GPo12(pGEc47) on citrate resulted in the standard fatty acid profile (Fig. 2f). When the cells were grown on octane, they showed the octane-induced fatty acid profile (Fig. 2e) first seen for octane-grown GPo1 (Fig. 2c), and the cells synthesized twice as much 18:1 as did the citrate-

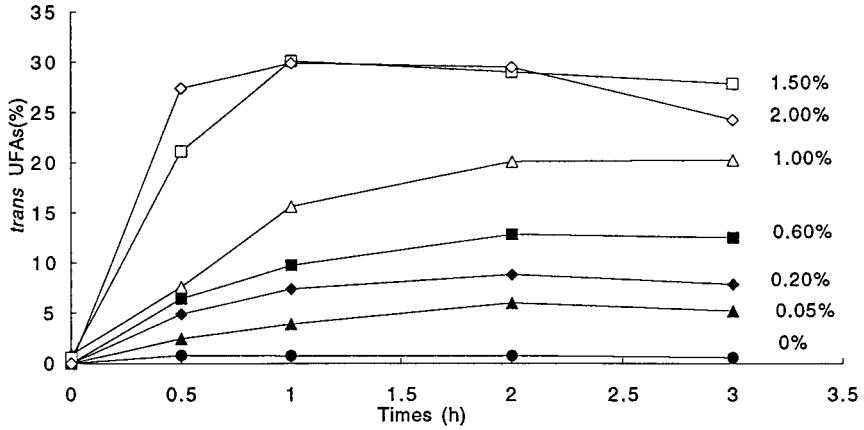

FIG. 7. Formation of *trans* unsaturated fatty acids (UFAs) by GPo12 after exposure of cells to different concentrations of 1-octanol in 20% (vol/vol) octane. The ratio of 1-octanol/octane (vol/vol) is shown on the right.

grown cells. As was the case for octane-grown GPo1 cells, GPo12(pGEc47) cells grown on octane also contained a significant amount of *trans* unsaturated fatty acids (Fig. 3b), which were present as trace constituents only in citrate-grown cells (data not shown).

Thus, introduction of the *alk* genes into the cured strain GPo12 caused the recombinant to mimic the standard and octane-induced fatty acid profiles also seen for the wild-type strain GPo1 during growth on citrate and on octane, respectively.

Effects of induction of *alk* genes with DCPK on the membrane composition of *P. oleovorans*. To further distinguish between the effects of the solvent and *alk* gene expression on the fatty acid profiles, the recombinants were grown on citrate in aqueous medium in the absence or presence of DCPK at a dilution rate of 0.1 h^{-1}. DCPK had no effects on the membrane lipid fatty acid compostion of GPo12. DCPK also had no effects on the growth of the controls GPo12(pGEc74) and GPo12(pGEc29), which contain only *alkST* and *alkBFGHJKL*, respectively, and therefore did not produce Alk proteins (Table 1 and Fig. 1). However, growth of GPo12(pGEc47) and GPo12(pGEc47Δ*B*), which contain both operons *alkST* and *alkBFGHJKL*, in the presence of DCPK did result in cell elongation, a decrease in the growth rate, and inactivation of the *alk* genes. The continuous inactivation of the *alk* genes in the presence of DCPK is illustrated in Fig. 4. In spite of the presence of tetracycline, cells which initially synthesized Alk proteins and were able to grow on octane started to lose this ability after continuous growth on citrate in the presence of DCPK for five to seven generations. As the *alk* genes were lost or inactivated, the amount of AlkB, which originally appeared as a clearly visible band after SDS-polyacrylamide gel electrophoresis of whole-cell lysates, decreased and could no longer be detected after 17 to 20 generations (Fig. 5).

Although these phenomena complicated experiments on the effects of the *alk* gene expression, it was possible to compare the fatty acid profiles following induction of the *alk* genes with DCPK. Figure 6a and b show the fatty acid composition of GPo12(pGEc47) in the presence and absence of DCPK, respectively. The fatty acid composition of the uninduced cells remained stable for the whole cultivation (Fig. 6b), whereas the fatty acid composition of the induced GPo12(pGEc47) recombinant changed during the cultivation (Fig. 6a). As long as the *alk* genes remained fully active, their induction and expression resulted in an increase in 18:1(11c) and 16:1(9t), compensated for by a decrease in 16:0, 16:1(9c), and 17:cy, similar to the findings for the cells grown on octane (Fig. 2e). As the *alk* genes were lost or inactivated (Fig. 4), the fatty acid composition of the DCPK-induced cells approached and finally reverted to that of noninduced cells (Fig. 6b).

As expected, the fatty acid compositions of DCPK-induced GPo12(pGEc74) and GPo12(pGEc29) remained stable and were identical to that of the uninduced GPo12(pGEc47) (data not shown), indicating that alterations in the fatty acid composition as a result of DCPK induction required the functions of both *alkST* and *alkBFGHJKL* sequences.

Effects of expression of *alkB* gene on fatty acid composition of *P. oleovorans*. The experiments for which the results are shown in Fig. 6a and b showed that the cells modified their membrane lipid fatty acid composition even in the absence of octane as long as there was expression and induction of the *alk* genes. To test whether *alkB* has a role in this process, these experiments were repeated with GPo12(pGEc47Δ*B*), a strain which does not produce AlkB because of a 528-bp deletion in *alkB* (38) but which does express downstream *alk* genes (38 and our unpublished observations).

Basically, the fatty acid composition of GPo12(pGEc47Δ*B*) was not affected by induction with DCPK (Fig. 6c and d) and closely resembled that of the uninduced GPo12(pGEc47) containing an intact *alkB* gene (Fig. 6b). Thus, the fatty acid composition changes seen in Fig. 6a were mainly associated with the induction and expression of *alkB*. These include changes in 16:0, 18:1(11c), and 16:1(9c). Changes seen in the formation of 16:1(9t) and 17:cy as a result of DCPK induction of GPo12(pGEc47) were also seen to a lesser extent for induced GPo12(pGEc47Δ*B*). These changes may be associated with expression of other DCPK-inducible *alk* genes.

Effects of 1-octanol on fatty acid composition of *P. oleovorans*. When induced, the alkane hydroxylase of *P. oleovorans* GPo1 converts octane to 1-octanol (Fig. 1) both in batch cultures (up to 1.5% [vol/vol] 1-octanol is formed in the octane phase in the stationary phase [28]) and in continuous cultures (results not shown). To test whether 1-octanol caused changes of fatty acid composition during growth of *P. oleovorans* on octane, we monitored the fatty acid composition of GPo12 after exposure of the cells to different concentrations of 1-octanol in the presence of 20% (vol/vol) octane.

The overall fatty acid composition of GPo12, such as the ratio of saturated to unsaturated fatty acids and the mean acyl chain length, remained unchanged. However, *trans* unsatur-

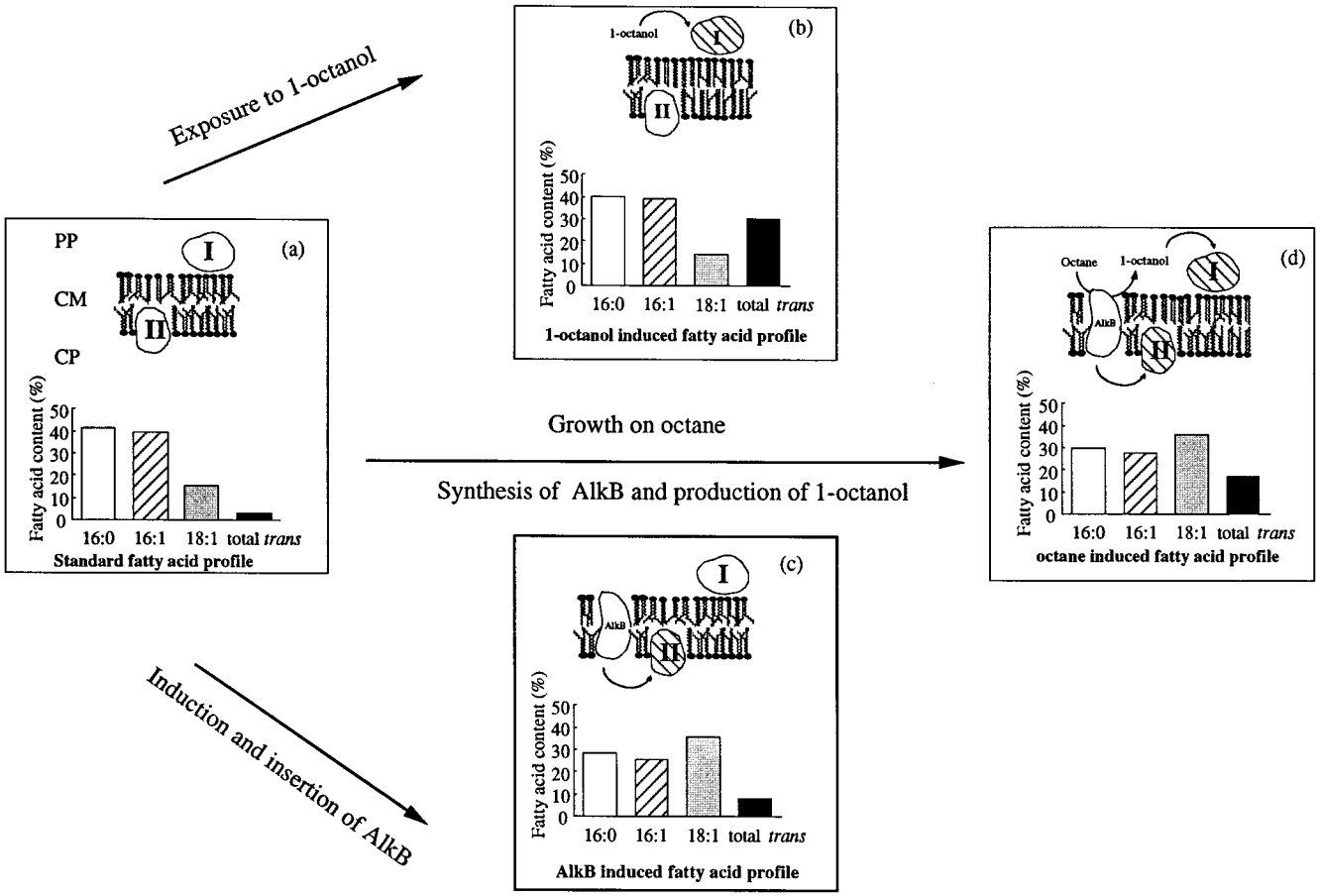

FIG. 8. Model for modification of fatty acid composition during growth of *P. oleovorans* on octane. Changes of the fatty acid composition from the standard fatty acid profile observed for the citrate-grown cells (a) to the octane-induced profile (d) during growth on octane occur mainly via two different processes: (i) 1-octanol activates the isomerization of *cis* to *trans* unsaturated fatty acids by a periplasmic isomerase (I), increasing the amount of *trans* unsaturated fatty acids and resulting in the 1-octanol-induced profile (b); (ii) induction of the *alk* genes results in insertion of AlkB into the cytoplasmic membrane, which affects the fatty acid synthesis and modification machinery (II), lengthening the mean acyl chain by increasing fatty acid 18:1 twofold and resulting in the AlkB-induced profile (c). Growth on octane induces the *alk* genes. This results in insertion of AlkB and activation of system II. It also results in a complete alkane hydroxylase (AlkB, AlkG, and AlkT [see the legend to Fig. 1 for abbreviations]) and production of 1-octanol. The isomerase I is activated both by the 1-octanol so formed and, to some extent, by expression of other *alk* genes. The activation of systems I and II leads to the octane-induced fatty acid profile. PP, periplasm; CM, cytoplasmic membrane; CP, cytoplasm.

ated fatty acids [16:1(9t) and 18:1(11t)] were formed at the expense of their *cis* isomers: the maximum amount of 16:1(9t) was about 27% of the total fatty acids, while 18:1(11t) amounted to less than 3% of the total fatty acids. Figure 7 shows that significant amounts of *trans* unsaturated fatty acids were observed within 0.5 h after exposure of cells to 1-octanol. The total amount of *trans* unsaturated fatty acids increased with increasing exposure time and 1-octanol concentration and reached a maximum of about 30% of the total fatty acids when the cells were exposed to 1.5% (vol/vol) 1-octanol for 1 h. These results show that 1-octanol can contribute significantly to the increase in *trans* unsaturated fatty acids during growth of *P. oleovorans* on octane.

DISCUSSION

As shown schematically in Fig. 8, during growth on octane, the membrane phospholipid fatty acid composition of *P. oleovorans* GPo1 changed from a standard profile (Fig. 8a) to an octane-induced profile (Fig. 8d), which is characterized by an increased content of fatty acid 18:1 and the formation of *trans* unsaturated fatty acids. The octane-induced profile was not seen for the OCT-plasmid-cured strain (GPo12) grown in the presence of octane, indicating that the octane-induced profile cannot be due to direct effects of octane on the cells. Instead, the octane-induced profile resulted from the induction and expression of the *alk* genes. The increase of 18:1 was due to effects associated with the synthesis of AlkB (Fig. 8c), while the formation of *trans* unsaturated fatty acids was due to effects associated with the conversion of octane to 1-octanol by the AlkB-containing alkane hydroxylase (Fig. 8b) and, to a lesser extent, the effects of expression of other *alk* genes.

Membrane incorporation of AlkB. When the *alk* genes are induced with octane (or DCPK), AlkB, a 46-kDa protein which is firmly embedded in the cytoplasmic membrane via six transmembrane helices (38), is produced. In the steady state, AlkB accounts for 25 to 30% of the total cytoplasmic membrane protein (12). Insertion of large amounts of AlkB into the cytoplasmic membrane probably affects the structure and physical characteristics of the *P. oleovorans* membrane, which may well influence membrane lipid synthesis, leading to changes in the membrane lipid composition. This notion is supported by the recent finding that membrane lipid synthesis in some *E. coli* recombinants is stimulated by *alkB* induction (33). Similar modifications of membrane composition as a result of the

incorporation of membrane proteins have also been described by Weiner and his coworkers (40), who found that overproduction of the membrane-bound fumarate reductase of *E. coli* resulted in alteration of both fatty acid and phospholipid headgroup compositions.

Modifications of the membrane composition could, in turn, facilitate the incorporation of membrane proteins. We have found that there is an increase in the content of unsaturated fatty acids, especially 18:1 (Fig. 2c and e) as the *alk* genes are induced and AlkB is produced. An increase in the mean acyl chain length is expected to increase the membrane thickness. The matching between the length of the hydrophobic part of membrane proteins and lipid hydrophobic thickness can influence the activities of different membrane proteins (2, 22, 24). Moreover, phosphatidylethanolamine with *cis* unsaturated fatty acyl chains is known to favor the formation of nonbilayer lipid phases in membranes under physiological conditions (7). Such transitory nonbilayer structures might be required to minimize packing defects around the irregular surfaces of membrane proteins (36), to activate membrane-bound enzymes (5, 23, 32), and to optimize membrane functions such as facilitated membrane transport (7). Thus, the observed changes could facilitate the insertion or function of membrane-bound Alk proteins.

AlkB-induced fatty acid profile. Synthesis of large amounts of AlkB resulted in an increase in the 18:1 content (Fig. 8c), which was presumably due to the activities of enzymes involved in fatty acid and phospholipid syntheses. The entire set of activators and enzymes which might be involved in such altered fatty acid synthesis is indicated as system II in Fig. 8. With *E. coli* as a paradigm, the most significant determinants of fatty acid chain length appear to be competition between the rate of elongation by the condensing enzymes, the supply of malonyl coenzyme A, and the utilization of acyl-acyl carrier proteins by acyltransferase (6, 17, 31, 35). The most likely candidates for the mediation of the response are the cytoplasmic membrane-bound acyltransferases. These membrane enzymes are able to alter their activities and substrate specificities in response to changes in the membrane environment caused by changing growth temperatures (6, 17, 31, 35). Thus, alterations of the membrane environment as a result of the insertion of a large amount of membrane-bound AlkB could also directly or indirectly affect these membrane-bound enzymes, thereby changing the membrane lipid composition. However, the influence of AlkB-mediated membrane changes on other enzymes of fatty acid synthesis cannot be excluded. For example, β-keto-acyl-acyl carrier protein synthase II (*fabF*) was found to be the sole enzyme responsible for increasing the amount of 18:1(11c) when *E. coli* was grown at a lower temperature (31). Whether the similar increase in 18:1(11c) on induction of the *alk* genes in *P. oleovorans* is due to the same mechanism remains a question.

1-Octanol-induced fatty acid profile. Exposure of the cells to 1-octanol made a major contribution to the isomerization of *cis* to *trans* unsaturated fatty acids in the cells, resulting in an octanol-induced profile (Fig. 8b). The enzyme system responsible for the isomerization is shown as system I in Fig. 8. It is known that 1-octanol intercalates between the phospholipid acyl chains by anchoring the hydroxyl group in the lipid-water interface region, which perturbs the lipid bilayer and increases the membrane lipid fluidity (30). Conversion of *cis* unsaturated fatty acids to their *trans* isomers results in an decrease in membrane fluidity (6). We have previously reported that the lipid transition temperature of octane-grown cells is 24 to 30°C, much higher than that of citrate-grown cells (6°C), indicating that growth of cells on octane results in a decrease in membrane fluidity, which can compensate for the increase in membrane fluidity engendered by 1-octanol (4). Recently, it has been shown that the resistance of several *Pseudomonas* strains to toxic organic solvents, such as phenolic compounds (20), toluene, styrene (19), and shorter *n*-alkanes ($C \leq 7$) (unpublished observations), is also accompanied by isomerization of *cis* unsaturated fatty acids to their *trans* isomers. Thus, formation of *trans* unsaturated fatty acids, which have not been found in most other bacteria (e.g., *E. coli*) appears to play an important role in regulating cell membrane fluidity in *Pseudomonas putida,* in addition to the well-known modulation of the mean acyl chain length and the degree of fatty acid unsaturation, generally seen for other bacteria.

Octane-induced fatty acid profile. On the basis of the model shown in Fig. 8, growth of *P. oleovorans* on octane is expected to have the following effects. First, AlkB is produced and inserted into the cytoplasmic membrane as a result of induction of the *alk* genes with octane. This activates system II, which results in an increase in the fatty acid 18:1 content (Fig. 8c). At the same time, other *alk* genes are also induced, and octane is oxidized to 1-octanol by the AlkB-containing alkane hydroxylase (Fig. 1). Exposure of the cells to 1-octanol activates system I, which causes isomerization of *cis* to *trans* unsaturated fatty acids (Fig. 8b). In addition, induction of other *alk* genes might also affect system I. The combination of these effects results in the octane-induced fatty acid profile (Fig. 8d), seen for *P. oleovorans* grown on octane.

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